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Brief Research Communication

Exclusion of CAG/CTG Trinucleotide Repeat Loci Which Map to Chromosome 4 in Bipolar Disorder and Schizophrenia

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The hypothesis that expanded trinucleotide repeats contribute to the pathogenesis of schizophrenia and bipolar disorder has been recently supported by three independent studies which have shown that patients with either disorder tend to have larger CAG/CTG repeat expansion detection products than controls. In an attempt to identify the specific expanded CAG/CTG locus or loci which are associated with schizophrenia and bipolar disorder, we determined the repeat size at CAG/CTG loci mapping to candidate regions for psychosis. In this study we report our findings from eight loci which map to chromosome 4. We conclude that these loci are unlikely candidates for CAG/CTG repeat expansion in schizophrenia and bipolar disorder. Am. J. Med. Genet. 74:204–206, 1997. © 1997 Wiley-Liss, Inc.

KEY WORDS: trinucleotide repeats; chromosome 4; schizophrenia; bipolar disorder; CAG repeats

INTRODUCTION

Several recent studies have shown that in families transmitting either schizophrenia or bipolar disorder, the age at onset of the disorder becomes younger in succeeding generations [reviewed in O'Donovan and Owen, 1996]. One explanation for these findings is that the transmission of both disorders displays true anticipation. If this explanation is correct, it suggests that an expanded trinucleotide repeat mechanism is involved in the genetic transmission of both schizophrenia and bipolar disorder, although ascertainment bias remains an alternative explanation [Asherson et al., 1994; Yaw et al., 1996].

Recently the dynamic mutation hypothesis has received strong support from molecular genetic studies. In four studies from three independent groups, the distribution of maximum size of the trinucleotide repeat CAG (or CTG) was significantly larger in the DNA of individuals with schizophrenia [O'Donovan et al., 1995, 1996; Morris et al., 1995; Vincent et al., 1996] compared with controls, and similar findings have been observed in bipolar disorder [O'Donovan et al., 1995, 1996; Lindblad et al., 1995]. One study of bipolar disorder failed to find such a difference, although the numbers were relatively small and therefore this may be due to sampling variance [Vincent et al., 1996]. All these studies used the method of repeat expansion detection (RED) [Schalling et al., 1993], which cannot reveal the particular expanded CAG/CTG repeat(s) associated with either disorder.

Unfortunately, the task of identifying the locus or loci containing the expanded repeat(s) is not simple and we have recently advocated the use of a genomic CAG repeat PCR screening set [Bower et al., 1996]. The main theoretical drawback of applying this CAG/CTG screening set is that the proportion of repeats which are within genes is as yet unknown. However, it appears that there is a relative exclusion of CAG repeats from intronic DNA [Stallings, 1994], and an analysis using the Gene Recognition and Analysis Internet Link [Xu et al., 1995] suggests that at least 40% of the CAG/CTG repeats in the screening set are likely to lie within coding sequences [O'Donovan and Guy, 1996]. These data, therefore, suggest that an approach based upon genomic repeats is at worst only slightly less efficient than an approach based upon cDNA [e.g., Margolis et al., 1996], and at best is considerably more efficient when one considers that the investigator is not required to clone genes.

At present, there are 338 loci in the screening set. Clearly an approach based upon the complete set using a classical case-control design would be formidable both in terms of genotyping and because of the reduction in power that would follow correction for multiple testing. We therefore developed a multistage approach to minimize these difficulties [Bowen et al., 1996]. First, genomic DNA from a large number of patients is

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screened with RED. Second, samples with RED product sizes within the size range of interest are genotyped at individual repeat loci. For our studies of schizophrenia and bipolar disorder, patients with RED products within the size range 60–90 repeats were chosen for genotyping because this is the range in which there is the greatest excess of patients with psychosis compared with controls [O'Donovan et al., 1996]. Finally, if large alleles are observed, a case-control study is undertaken using that marker. We calculated that in the initial phase, only eight DNA samples from patients need to be genotyped to achieve 95% power for detecting a expanded repeat associated with either disorder, thereby providing a considerable saving in genotyping [Bowen et al., 1996].

Using this approach, since all known pathogenic CAG/CTG repeats are polymorphic, we first excluded expansions at all known polymorphic CAG/CTG loci in the set [Gastier et al., 1996] in both schizophrenia and bipolar disorder [Bowen et al., 1996; Guy et al., in preparation]. However, it is likely that some of the other repeats in the screening set are actually polymorphic, because the polymorphism content was not determined if the repeat number was <7 [Gastier et al., 1996]. We therefore chose to expand our study and examine these remaining repeats in patients with either schizophrenia or bipolar disorder. Loci which map to candidate regions identified by linkage studies were given priority for analysis. In this study, we present the results from a screen of the CAG/CTG repeats which map to chromosome 4.

Chromosome 4 was chosen as a candidate for further investigation because of a recent report [Blackwood et al., 1996] of a single pedigree with significant evidence of linkage between bipolar disorder and chromosome 4 markers ($Z_{max} = 4.09$ at D4S394). Weaker evidence for a locus in this region ($Z_{max} = 1.89$) had previously been reported in a single pedigree transmitting a psychotic illness characterized mainly as shizoaffective disorder [Asherson et al., 1995]. In light of these findings we postulated that a pathogenic expanded repeat linked to chromosome 4 was responsible for the association between large CAG/CTG repeats and the psychoses.

Ten subjects with DSMIII-R [American Psychiatric Association, 1987] bipolar disorder and 10 subjects with DSMIII-R schizophrenia with maximum RED products of between 60–90 repeats were selected from the UK cohort of a RED study. All were Caucasians. Diagnoses were made with OPCRIT version 3.31 [McGuffin, et al. 1991], following a semistructured interview and examination of case notes. The sample included an affected member from the pedigree mentioned above [Asherson et al., 1994].

Primer pairs for GCT2C08, GCT4B02, GCT6B12, GCT6F01, GCT7C02, GCT10B09, GCT12H11, and GCT13F01 loci were obtained from the UK Human Genome Maping Project (HGMP) Resource Center. Five other loci from the screening set were excluded because their map positions placed them beyond even the broadest area of linkage. GCT17D01 and GCT15D08 are, respectively, 80 cM and 95 cM distal to D4S394 [Blackwood et al., 1996], and GCT16C02, GCT8D06, and GCT1B3 are, respectively, about 562 cR, 706 cR, and 703 cR distal on the integrated STS-based map of the genome [Hudson et al., 1996].

PCR amplification was carried out as described [Bowen et al., 1996]. PCR products were detected by hybridization with a radiolabelled CAG repeat oligonucleotide, following Southern blotting and hybridization as described [Bowen et al., 1996], except that the stringency of hybridization and washing varied between 42–65°C. Despite PCR bias towards smaller alleles, this method is not biased against the detection of the modest-sized repeat expansions that are within the range of interest here, and expanded Huntington disease alleles are easily seen (data not shown).

None of the markers except GCT10B09 was polymorphic in the patients. GCT10B09 yielded an allele which was present in 10 of 40 chromosomes analyzed. The size of this product was 146 bases. This represents a maximum number of 10 CAG repeat units under the assumption that any size difference observed was due to expansion of the CAG repeat. Only a single allele was found out of 40 chromosomes at each of the other seven loci. These were: GCT2C08 (five repeats), GCT4B02 (six repeats), GCT6B12 (five repeats), GCT6F01 (five repeats), GCT7C02 (six repeats), GCT10B09 (five repeats), GCT12H11 (five repeats), and GCT13F01 (six repeats).

Clearly, in our sample, there are no alleles that contain repeats within the putative size range of interest in bipolar disorder or schizophrenia [O'Donovan et al., 1995] or which are, by analogy with other CAG repeat diseases, consistent in size with a pathogenic effect. Therefore, expansions at these loci cannot explain the associations between large CAG/CTG repeats and either schizophrenia or bipolar disorder. Together with data from previous investigations [Bowen et al., 1996; Guy et al., in preparation], we have now examined and excluded expansions in all 12 of the CAG/CTG repeats in the screening set [Gastier et al., 1996] which have mapped locations [Hudson et al., 1996] consistent with linkage findings on chromosome 4 [Blackwood et al., 1996].

In complex diseases such as schizophrenia and bipolar disorder which are likely to be genetically heterogeneous, sample size and power are critical considerations [Bowen et al., 1996]. The number of subjects examined in this study is relatively small, and therefore it may be assumed that this study has low power to detect expanded repeats conferring susceptibility to either disorder. However, we selected subjects from much larger samples on the grounds that they are the most likely to carry expanded repeats related to the disorders [O'Donovan et al., 1996]. Consequently, the power in this study is much greater than would be achieved by random patient selection.

Even allowing for genetic heterogeneity, based upon the RED product distributions in patients and controls, we estimate the power using 10 probands to be >98% [Bowen et al., 1996]. Our estimate of power allows for genetic heterogeneity within each disorder and for different repeats to confer susceptibility to schizophrenia and bipolar disorder [Bowen et al., 1996]. It does not, however, allow for the possibility that more than one expanded CAG/CTG repeat confers susceptibility to

each disorder. If there are four distinct susceptibility loci containing expanded CAG/CTG repeats, two of equal frequency for each disorder, the power to detect an expansion at each locus falls to 84%, and the corresponding figure for six distinct susceptibility loci is 69%. It follows that our evidence for the exclusion of loci is dependent on the true number of expanded repeats that contribute susceptibility to schizophrenia and bipolar disorder. At present, we have no reason to assume that this number is greater than one, but if there are multiple loci containing expanded repeats that contribute to the etiology of psychosis, a further improvement in power can be achieved by further restricting the sample to probands from pedigrees that give evidence for linkage to chromosome 4.

Finally, we stress that although we effectively excluded (within the limits of the discussion above) all of the chromosome 4 loci in the published screening set as candidates for the CAG/CTG repeat expansion in schizophrenia and bipolar disorder, we have not excluded the possibility that the linkage data of Blackwood et al. [1996] are due to an unknown CAG/CTG expansion because the screening set is incomplete, with only $\frac{1}{2}$–$\frac{1}{3}$ of the total expected number of CAG/CTG repeats of five or more units represented.

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